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Collection, Chemical Fractionation, and Mutagenicity Bioassay of Ambient Air Particulate

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16. ABSTRACT

The influence of industrialization and consequent increased concentration of urban particulate matter on the incidence of cancer has long been a concern (Kotin and Falk, 1963; Carnow and Meier, 1973). The first bioassays used to evaluate complex ambient air samples were whole-animal carcinogenesis bioassays (Leiter et al., 1942; Hueper et al., 1962). In these studies, organic extracts of urban particulate matter were found to be carcinogenic in rodents. Such organic extracts have also been shown to transform rodent embryo cells in culture (Freeman et al., 1971; Gordon et al., 1973). Carcinogenic polycyclic aromatic hydrocarbons (PAH), such as benzo(a)pyrene, were detected in these extracts; however, these compounds did not account for all of the carcinogenic activity reported.

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by

Michael Waters, Joellen Lewtas Huisingh, and Larry Claxton
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Alan Kolber, Thomas Wolff, Thomas Hughes, and Edo Pellizzari
Research Triangle Institute
Research Triangle Park, NC

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HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NC 27711

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COLLECTION, CHEMICAL FRACTIONATION, AND MUTAGENICITY BIOASSAY OF AMBIENT AIR PARTICULATE

Alan Kolber, Thomas Wolff, Thomas Hughes, Edo Pellizzari,
and Charles Sparacino
Research Triangle Institute
Research Triangle Park, North Carolina

Michael Waters, Joellen Lewtas Huisingh, and Larry Claxton
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina

INTRODUCTION

Our industrial society has created thousands of synthetic xenobiotics to support our modern lifestyle. Many of these substances, and their by-products, enter our atmosphere in the form of vapor-phase and particulate pollutants that can be ingested by respiration and skin contact. The insults to human health and the ecosystem from these airborne organic pollutants are due mainly to polycyclic organic matter, specific industrial emissions such as halogenated hydrocarbons, and end-use chemicals such as pesticides (Fishbein, 1976). Polycyclic organics associated with air particulates are believed to result primarily from incomplete combustion of organic matter (Møller and Alfheim, 1980). These carcinogenic components, (benzo(a)pyrene (B[a]P), benz(a) anthracene) and other polycyclics have been well characterized chemically (Shubik and Hartwell, 1969). As population and industrial activity increase, the growing health hazards associated with ambient air pollution must be further assessed and evaluated.

Air pollutants are chemically complex environmental mixtures, whose compositions vary geographically with local industrial activity and weather conditions. Seasonal variations in chemical composition and mutagenicity of organic extracts of air particulate have been documented in Oslo, Stockholm, and New York City (Møller and Alfheim, 1980; Löfroth, 1980; Daisey et al., 1979). All three communities exhibited higher mutagenicity during the winter months, and in each case, a major contributing factor was polynuclear aromatic hydrocarbons (PAH). These indirect-acting mutagens, which require metabolic activation, were produced from combustion of heating oil.

Ambient-air particulate matter has been estimated at 56×10^{13} g/yr, total global load, including natural pollutants such as dusts, agricultural particulate, and others. The man-made (anthropogenic) global load is estimated to be 28×10^{13} g/yr. Vapor-phase pollutant organics are believed to represent 60 to 90% of the total organic load; the remainder is organics adsorbed onto particulate matter (Hidy and Brock, 1970; Duce, 1978).

Chemical monitoring and analysis of air pollutants have been conducted for many years, but only within the last decade has biotesting technology improved enough to permit simultaneous chemical and biological characterization. However, chemical-analytical expertise exceeds the present biological capabilities, both qualitatively and quantitatively (Hughes et al., 1980).

In this study, air particulate was collected, size-fractionated, solvent-extracted and fractionated into chemical classes, which were then characterized by GC/MS/computer analysis. These chemical class fractions were then biotested for mutagenic activity using the Ames/Salmonella bacterial mutagenicity assay. Vapor-phase organic pollutants were also collected and tested. However, no quantitative method was available to adequately measure the mutagenicity of the vapor-phase components; consequently, we are presently developing a bioassay protocol capable of quantifying the mutagenicity of vapor-phase substances. It should be noted that mutagenicity or carcinogenicity is by no means the only significant health hazard that could be presented by air pollutants. As additional in vitro bioassay capabilities are developed, other potential toxicity parameters will be examined, such as neurotoxicity and lung toxicity.

METHODS

Collection of Air Particulate and Vapors

Ambient air particulate was collected by the Maxisampler, a high-volume sampling device constructed by the Battelle Corporation after the design by Henry and Mitchel (1978). This device can sample 20,000 m of air in a 24-h period and can collect particulate matter within the respirable range in three size fractions (< 1.7 μm, 1.7 to 3.5 μm, and > 3.5 μm), using impactor plates and electrostatic precipitation. A mechanical diagram of the sampler is shown in Figure 1. After collection, the plates were sealed in a transportable container; and the particulate material was removed and characterized morphologically by scanning electron microscopy. Figure 2 is a typical scanning electron micrograph of ambient air particulate. Air particulate was sampled at five U.S. locations: Elizabeth, N.J.; Upland, CA; Lake Charles, LA; Houston and

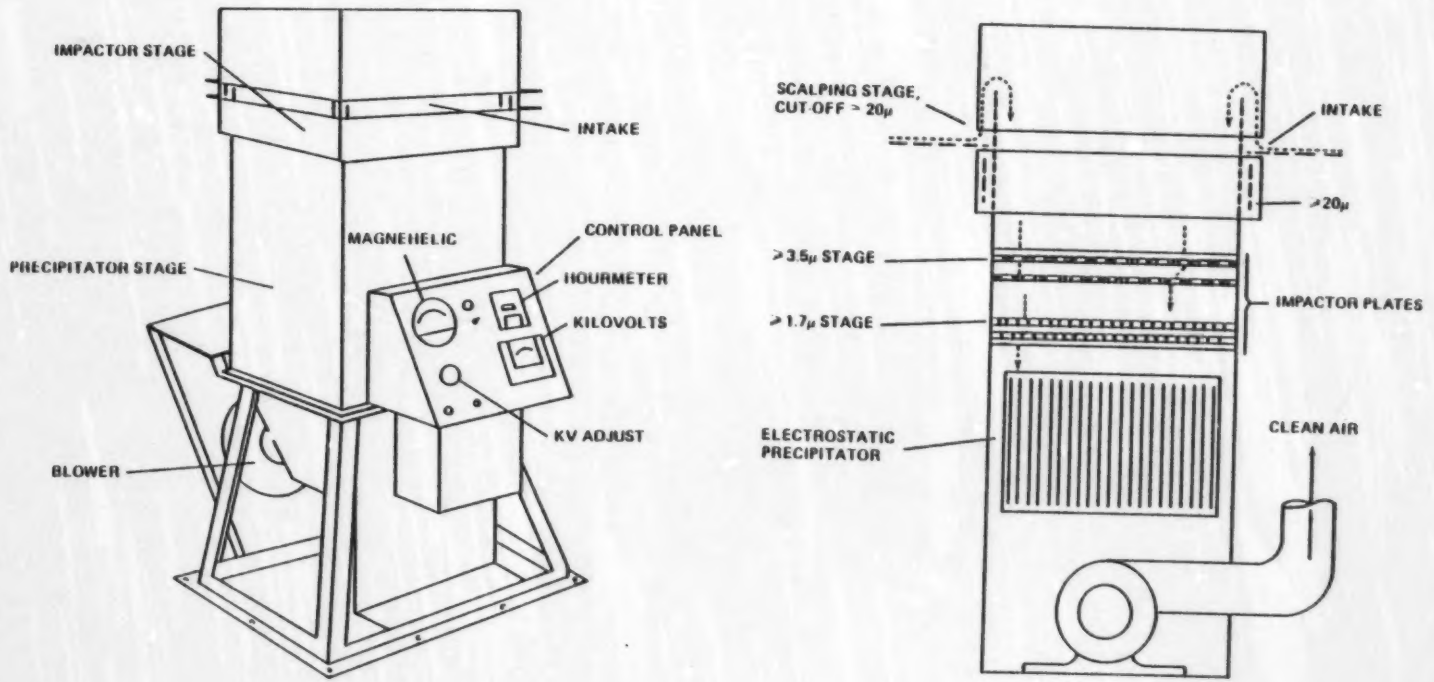


Figure 1. Schematic of the Battelle massive air volume sampler.

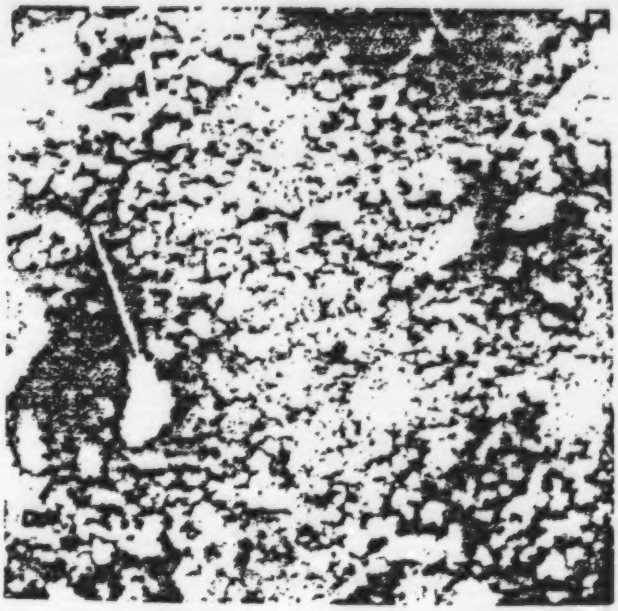


Figure 2. Scanning electron micrograph of ambient air particulate from Lake Charles, LA (> 3.5 μm).

Beaumont, TX. Table 1 summarizes the sampling variables for Elizabeth, N.J.

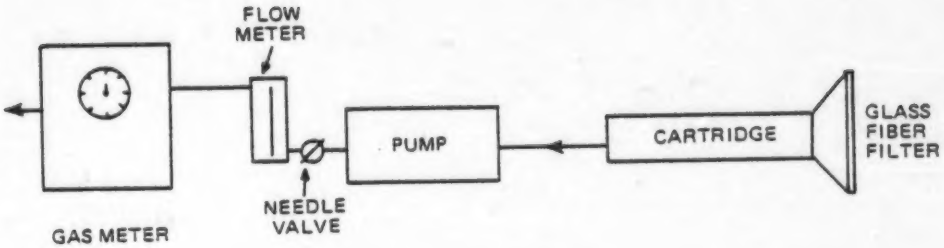
Vapor-phase substances were sampled with cartridges containing Tenax polymeric sorbent. Each cartridge was sealed and later thermally desorbed into cryogenic traps (see Figure 3). This frozen sample was then tested in the Ames/Salmonella assay. Negative results were always obtained, but this may be due to an inadequate testing procedure.

Chemical Fractionation of Ambient Air Particulate

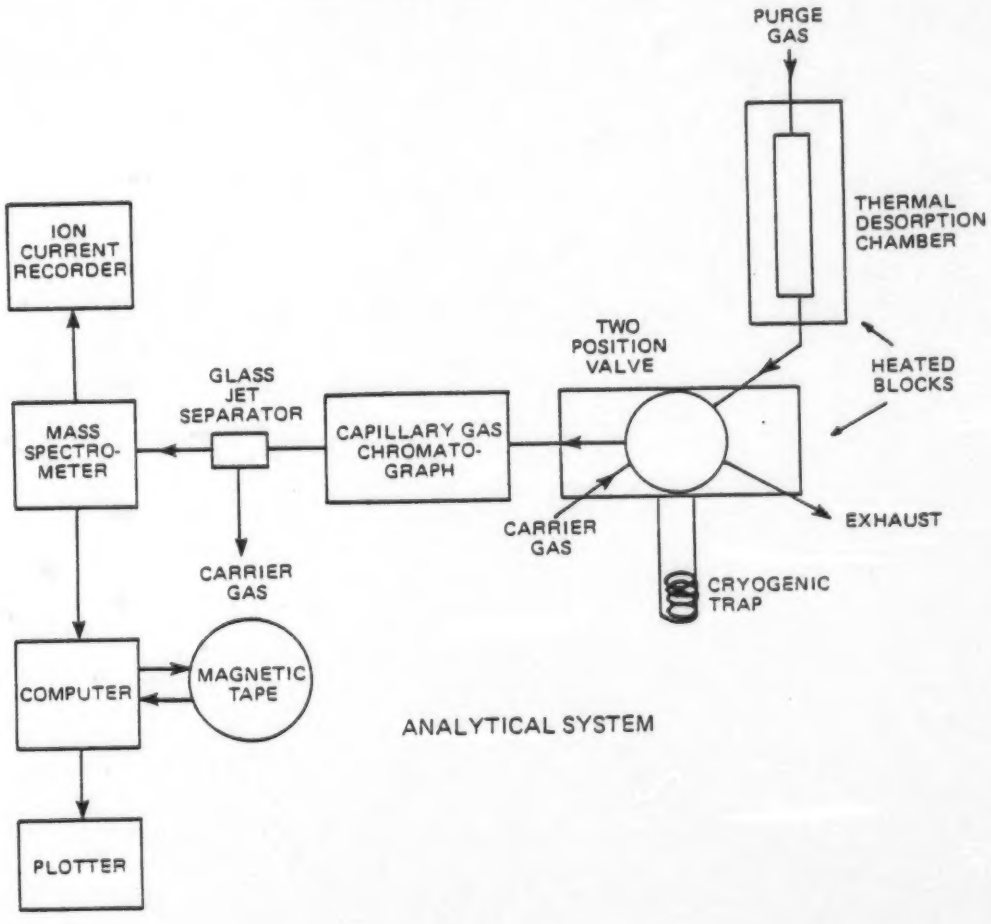
An initial chemical fractionation scheme developed by Pellizzari et al. (1978) that generated 13 polar and nonpolar chemical classes was utilized to chemically fractionate the size-fractionated air particulate. However, the chemical differences among the fractions were small, and dividing the crude particulate into 13 fractions resulted in sample sizes of less than 5 mg, which

Table 1. Sampling Variables for Particulate Organics in Ambient Air in Elizabeth, NJ

Sample Type	Date	Temp. °C (°F)	Sample Vol. (l)	Duration of Sampling (min)	Remarks		
					Time	Relative Humidity	Wind Dir/Vel. kts (m/s)
Vapor-phase organics	9/19-20/78	17 (62)	646	1,390	1515-1425	90%	NE/5-10 kts (2.5-5)
	9/20-21/78	23 (74)	1,211	1,455	1445-1500	91%	SW/10 kts (5)
	9/21-22/78	20 (68)	1,044	1,265	1515-1220	90%	NE/10 kts (5)
	9/22-23/78	20 (68)	1,093	1,335	1245-1100	88%	NE/ 5 kts (2.5)
	9/23-24/78	16 (60)	1,279	1,690	1120-1530	75%	SE/10 kts (5)
Particulates	9/19-22/78		81,211,000	4,560	0805-1200		
	9/22-25/78		78,892,000	4,430	1210-1400		
	9/25-28/78		70,246,000	4,060	1405-0745		



VAPOR COLLECTION SYSTEM



ANALYTICAL SYSTEM

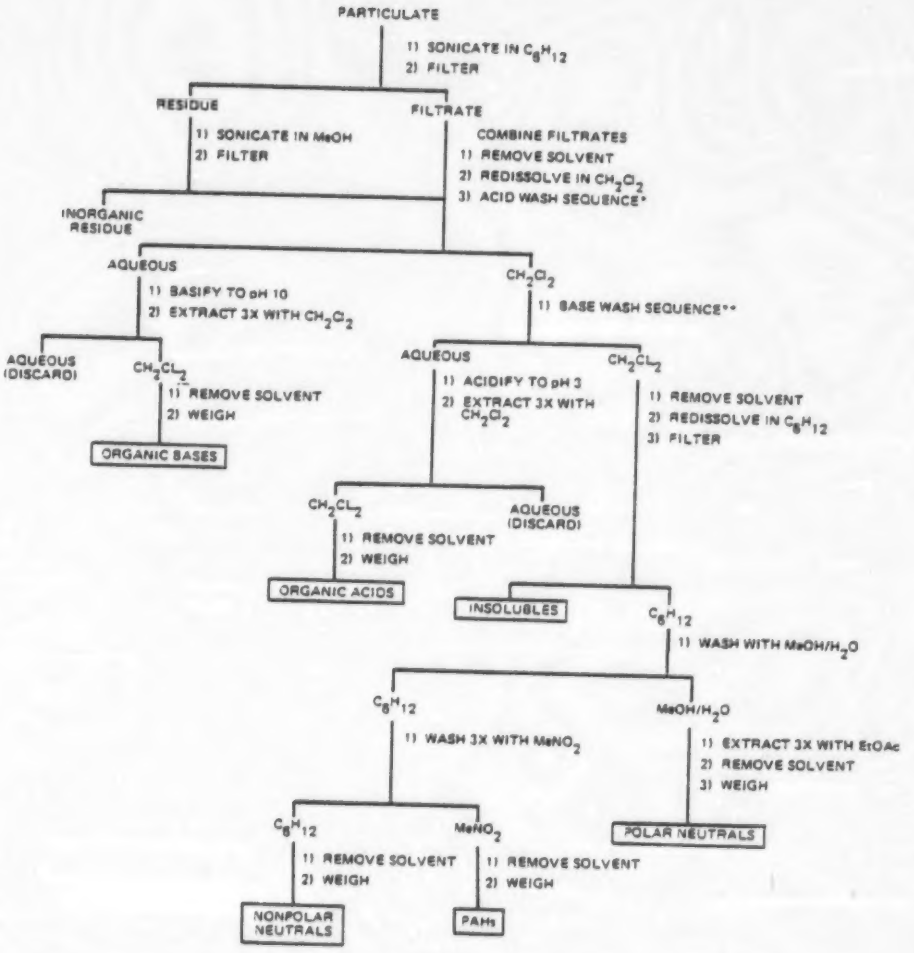
Figure 3. Vapor collection and analytical systems for analysis of organic vapors in ambient air.

limited adequate bioassay and chemical identification. The scheme was modified to generate six chemical classes: acids, bases, PAHs, polar neutrals, nonpolar neutrals, and insolubles (see Figure 4).

Air particulate (~1.0 g) was subjected to ultrasonic treatment in 100 ml cyclohexane (Burdick-Jackson) for 30 min and then filtered through a Teflon (DuPont) filter (0.5-μm pore size). The filtrate was evaporated to dryness using a rotary evaporator; the solids retained by the filter were dissolved in 100 ml methanol (Burdick-Jackson), and sonicated and filtered through the Teflon filter; and the filtrate was taken to dryness. The filtrates were combined, dried, weighed, and redissolved in methylene chloride (CH_2Cl_2). The solution was spiked with internal standards to allow for quantification and to serve as a quality control parameter for the overall partition/analysis scheme. The standards used were quinoline-d7 (organic base), phenol-d5 (organic acid), and anthracene-d10 (PAH).

This CH_2Cl_2 solution was extracted twice with equal volumes each of 10% sulfuric acid (H_2SO_4) and then once with 20% H_2SO_4. The aqueous phases were combined and washed with CH_2Cl_2, and this CH_2Cl_2 phase was combined with the original CH_2Cl_2 solution. The aqueous phase was cooled (ice bath), adjusted with 25% sodium hydroxide (NaOH) to pH 10, and extracted three times with CH_2Cl_2 to generate the "organic bases." The aqueous phase was discarded. The original CH_2Cl_2 solution was extracted three times with 5% NaOH, and the aqueous phases were combined and washed with CH_2Cl_2. The CH_2Cl_2 solution then was combined with the original CH_2Cl_2 solution. The NaOH phases were placed in an ice bath and acidified to pH 3 with 20% H_2SO_4 and extracted three times with CH_2Cl_2 to generate "organic acids." The remaining aqueous phase was discarded.

The original CH_2Cl_2 phase was evaporated to dryness, reconstituted in cyclohexane, and filtered through a Teflon filter (0.5 μm). The cyclohexane filtrate was extracted three times with an equal volume of methanol:water (4:1) solution; the methanol: water extract was then concentrated, and the water extracted three times with ethyl acetate. The solvent was removed to generate "polar neutrals." The cyclohexane phase was extracted three times with an equal volume of nitromethane; the nitromethane phases were then combined and evaporated to dryness to generate the PAHs. The cyclohexane phase was evaporated to dryness to generate the "nonpolar neutrals" fraction.



*Acid Wash Sequence: 2 X with 10% H_2SO_4, 1 X with 20% H_2SO_4
**Base Wash Sequence: 3 X with 1 N NaOH.

Figure 4. Fractionation scheme for ambient air particulate organics.

This procedure is a modification of a procedure described by Lee et al. (1976). The partition scheme has been validated for efficiency and presence of spillover of chemicals from one class to another, using mixtures of deuterated known substances representative of the individual chemical classes. Known mutagens

were included in the mixture, and the recovery of mutagenic activity before and after fractionation was monitored by the Ames/Salmonella bacterial mutagenicity bioassay.

Mutagenicity Bioassay

The plate-incorporation Ames/Salmonella mutagenicity assay was performed as described by Ames et al. (1975), with and without S-9 rat liver microsomal activation preparation. All assays were conducted with standard mutagens (positive controls) and solvent (negative) controls (samples were exchanged into dimethylsulfoxide [DMSO]). For revertant selection, minimal Vogel-Bonner medium E, supplemented with 1.6% Difco bacto agar and 2% dextrose, was used for base agar layers (25 ml, poured automatically). A 2.5 ml soft agar overlay containing bacteria, minimum amounts of histidine and biotin, and sample was routinely used.

To prepare S-9 from rat liver, the following procedure was used: Male Charles River rats, strain CD_1 (200 ± 20 g), served as the source of liver material. The animals were housed in suspended cages for one week before induction. Food and water were given ad libitum. Induced rat liver (from at least three rats) was obtained from rats injected intraperitoneally on day one with 500 mg/kg of Aroclor 1254 in Mazola Corn Oil (0.5 ml of a 200 mg/ml for a 200 g rat). On day five, the animals were sacrificed and their livers removed. These were immersed in cold, sterile 1.15% KCl, washed two times, and blotted dry with sterile paper. The livers were weighed, minced, brought to 200 mg/ml with 0.25 M sucrose, and homogenized (on ice) with four strokes of a cold Potter-Elvenhjem apparatus with a Teflon pestle. The homogenate was centrifuged for 10 min at 9,000 x g, the lipoprotein layer aspirated off, and the protein concentration of the liver supernatant adjusted (after protein assay) to 30 mg protein/ml with 0.25 M sucrose stock solution. The stock solution was checked for sterility, quick-frozen in small aliquots (2 to 5 ml), and stored for a maximum of two months at -80°C. For the assay, an aliquot of stock solution was slow-thawed and adjusted to an appropriate concentration of protein in 0.25 M sucrose. NADPH was added at 320 µg/plate. Each batch was checked against known positive controls using the plate-incorporation assay. The optimal S-9 protein concentration for mutagenicity was determined by testing with B(a)P and 7-12 dimethylbenzanthracene (DMBA).

Agar diffusion well technique. A modification of the bacterial mutagenicity test was developed to screen for chemical fractions of air particulate organics when sample size was limited. In this test, the sample(s) and S-9 microsomal preparation were placed in wells cut in the agar. Soft agar then was added to fill the well, and after solidification, the bacterial overlay

in 2.5 ml soft agar was poured over the entire surface. Results were interpreted as follows: a direct acting mutagen was observed as a ring of colonies around the sample well; a promutagen was observed as a line of colonies between the sample and S-9 wells, and toxicity resulted in a clear zone around the sample well.

This qualitative bacterial mutagenicity test was verified with known mutagens, and its sensitivity was shown to be similar to that of the spot test. Optimum size, number, configuration, and spacing of wells, as well as amount of S-9 rat liver microsomal preparation required per well, were determined. A configuration suited to general screening included a center S-9 well and four surrounding sample and control wells, as illustrated in Figure 5. Advantages of this test over the spot test included ability to 1) measure multiple parameters (dosage, activation requirements, toxicity, and mutagenicity) on a single plate, thereby greatly extending the amount of information obtainable from the available sample; 2) determine positive, negative, and solvent controls on the same plate as the test compound; 3) conduct dose-response and multiple-compound testing on a single plate; 4) eliminate adsorption of test compound by the filter disc; 5) eliminate runoff of liquids onto the agar; and 6) reduce potential loss of mutagenic activity by chemical hydrolysis.

A priority scheme for biological testing was also developed, and was based on total size of each chemical-class fraction:

1) For > 10 mg of sample, the plate-incorporation method was used, with five nontoxic dose levels (at least in duplicate) in the following order: TA98, TA100, TA1535, TA1537, and TA1538. Toxicity was tested at the highest dose only. Preferred initial dose ranges were 1000, 500, 250, 100, and 10 µg/plate. The assay was performed first with, and then without, Aroclor-induced S-9 (3.0 mg/plate).

2) For < 10 mg of sample, the agar well diffusion test was used at two concentrations (500 and 100 µg/plate), except for PNA fractions which were tested using poured plates (500 µg/plate, with Aroclor-induced S-9 and TA98. Duplicate plates were assessed in the following order: TA98, TA1535, TA1537, TA1538, and TA100. Sample was diluted to one concentration for all tests; to obtain lower test concentrations, a smaller sample volume per well was used. Toxicity was tested at 500 µg/plate with TA98 and TA1535, using 1000 colonies/plate.

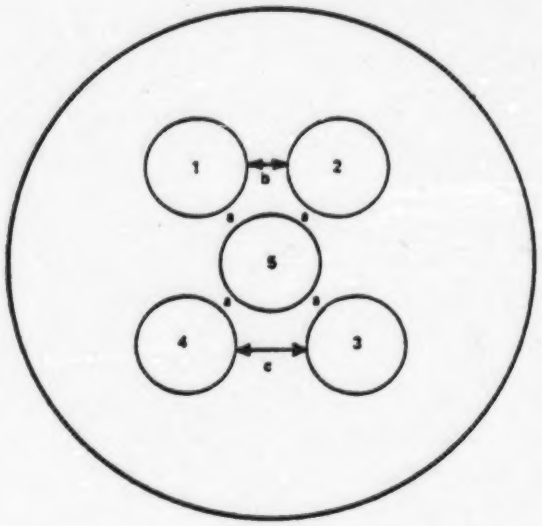


Figure 5. Well testing configuration for bacterial mutagenesis screening. The wells contain: 1) positive control promutagen (e.g., 100 µg of 2-anthramine in 100 µl solvent); 2) DMSO, solvent control (100 µl); 3) low concentration of sample (100 µg sample in 20 µl DMSO); 4) high concentration of sample (500 µg sample in 100 µl DMSO); 5) Aroclor-induced S-9 microsomal preparation ([3.0 mg protein] + NADPH [480 µg in 300 µl]). The distances are: a) 2.5 mm; b) 5.0 mm; c) 10.0 mm. The wells are 18.0 mm in diameter.

RESULTS

Collection, Extraction, and Fractionation

Sampling of 81 x 10^6 l ambient air required 76 h and generated 1.347 g of particulate, of which 180.1 mg were extractable organics, or 2.3 ng organic/l ambient air. Table 2 shows the total particulates collected for each size fraction from each sampling trip. Extractable organics varied from 2.5 to 15.3% of the particulate mass, and the smaller size fractions did not consistently contain the greatest amounts of organics (the 53% extractable organics for Beaumont, TX [< 1.7 µm] is probably incorrect, due to a weighing error). Table 3 illustrates the distribution of the extracted organic after chemical fractionation; 12 to 15% loss of organic matter was common during fractionation.

Table 2. Total Particulate Samples Collected

Sample Location	Particulate Size Range (μm)	Particulate Weight (mg)	Total Organics Extracted (mg)	% Extracted
Upland, CA	<1.7	666	33	5.0
	1.7 to 3.5	641	36	5.6
	>3.5	286	18	6.3
Houston, TX #1[a]	<1.7	337	19	5.6
	>1.7	790	86	10.9
Lake Charles, LA #1	<1.7	747	25	3.3
	>3.5	113	12	10.6
Elizabeth, NJ	<1.7	1347	180	13.4
	1.7 to 3.5	1302	120	9.2
	>3.5	1435	99	6.9
Lake Charles, LA #2	<1.7	2618	401	15.3
	1.7 to 3.5	488	15	3.1
	>3.5	548	27	4.9
Beaumont, TX	<1.7	285	151	53.0
	1.7 to 3.5	882	31	3.5
	>3.5	1701	42	2.5
Houston, TX #2	<1.7	3043	110	3.6
	1.7 to 3.5	1079	-	-
	>3.5	1353	-	-

[a]Particles > 1.7 μm were combined to generate sufficient sample for fractionation and biotesting.

Table 3. Fractionation of Organics Extracted from Ambient Air Particulate Samples

Sample Location	Size Range (μg)	Total Organics Extracted (mg)	Amount of Organics in Each Fraction (mg)					
			Acid	Base	Nonpolar Neutrals	PAHs	Polar Neutrals	% Recovery
Upland, CA	<1.7	32.7	2.9	1.0	6.5	1.6	15.8	85.0
	1.7 to 3.5	17.5	1.8	0.6	4.7	2.0	6.3	88.0
	>3.5	35.9	1.1	0.7	9.4	2.5	16.0	82.7
Houston, TX #1	<1.7	19.3	1.0	0.6	4.0	3.2	9.9	96.9
	>1.7	86.4	0.8	1.2	3.2	2.4	33.2	47.2
Lake Charles, LA #1	<1.7	24.6	7.2	0.1	6.1	2.1	3.7	78.0
	>1.7	11.9	1.7	0.1	3.9	2.3	2.4	87.4
Elizabeth, NJ	<1.7	180.1	8.5	0.5	86.2	11.1	34.6	78.2
	1.7 to 3.5	119.7	10.4	0.4	47.6	9.6	26.1	78.6
	>3.5	99.4	5.5	0.2	52.6	6.0	13.4	78.2
Lake Charles, LA #2	<1.7	401.2	170.2	16.3	49.2	7.1	152.6	98.6
	1.7 to 3.5	15.2	3.9	1.9	4.9	1.5	1.3	88.8
	>3.5	26.8	16.2	3.1	5.3	1.0	0.8	98.5
Beaumont, TX	<1.7	150.6	15.5	6.3	50.3	8.5	6.7	58.0
	1.7 to 3.5	30.7	6.8	0.6	12.2	2.2	8.9	100.0
	>3.5	42.1	10.4	0.4	21.3	4.8	3.8	96.7
Houston, TX #2	<1.7	110.2	20.4	2.8	20.9	12.2	38.0	85.6
	1.7 to 3.5	ND[a]	ND	ND	ND	ND	ND	ND
	>3.5	ND	ND	ND	ND	ND	ND	ND

[a]ND = not determined.

Mutagenicity

Significant mutagenicity was observed for various chemical fractions of the extracted particulate organics from some sites. A sample was considered mutagenic when it generated at least two times the number of revertants over spontaneous background for that bacterial strain. The PAH fraction was mutagenic at all five sites. The polar neutral and organic acids were mutagenic at four of the five sites, and the organic bases at three of the sites. The mutagenicity data on fractions available in quantities too low for the plate incorporation data were determined using the well test. Both indirect- and direct-acting mutagens were present in the complex mixtures and chemical fractions. An example of the mutagenicity data obtained during this study is given in Table 4.

Chemical Analysis

Chemical fractions from each of the organic extracts of the five geographical sites were analyzed by gas chromatography-mass spectrometry (GC/MS). Compounds identified from the analysis were matched against known toxic chemicals from the U.S. Health, Education, and Welfare Chemical Registry and other sources. Table 5 lists known mutagenic, carcinogenic, neoplastic, and teratogenic compounds identified. Many of these toxic compounds were found in the extracted organics from all sites for which chemical analysis was performed. Toxic PAH compounds were best represented.

DISCUSSION

This study was designed to develop and validate an integrated multidisciplinary approach to the study of genotoxic effects of ambient air pollutants. Engineering principles were employed to develop and test the massive air volume sampler used to collect and size-fractionate ambient air particulate. Analytical chemists developed and validated the cyclohexane-methanol extraction scheme for particulate organic matter and the acid-base extractive fractionation scheme to separate the crude organic extract into substituent chemical classes, which were then submitted to the biologist for mutagenesis testing. Such multidisciplinary approaches to the identification of bioactive chemical substituents of various complex environmental mixtures, including organics extracted from ambient air particulate matter, have been employed in recent studies, including those of Epler et al. (1978), Rao et al. (1980), Tokiwa et al. (1977), and others.

Table 4. Mutagenesis Data of Particulate Fractions from Lake Charles, LA (<1.7 μm)[a]

Sample Fraction	Doses (μg/plate)	HA Protein Concentration (mg)	TA100 +HA[b]	TA100 -HA	TA98 +HA[b]	TA98 -HA	TA1538 +HA[b]	TA1538 -HA	TA1537 +HA[b]	TA1537 -HA
Total organics	1000	3.0	507* ± 17	450* ± 21	127* ± 28	210* ± 70	152* ± 28	125* ± 12		
	500	3.0	327 ± 15	341 ± 30	74 ± 24	169* ± 12	60 ± 5	80 ± 0		
Polar neutrals[c]	500	3.0	401 ± 18	387 ± 2	129* ± 51	241* ± 6	117 ± 6	191* ± 12	67 ± 2	21 ± 11
	100	3.0	186 ± 63	256 ± 73	94 ± 23	155* ± 9	56 ± 8	74 ± 12	24 ± 10	23 ± 9
Acids[c]	500	3.0	326 ± 9	221 ± 91	82 ± 15	77 ± 17	59 ± 21	67 ± 11	62 ± 17	19 ± 10
	100	3.0	150 ± 95	168 ± 57	63 ± 20	89 ± 22	61 ± 5	52 ± 12	47 ± 21	39 ± 4
Bases	500	1.5	163 ± 106	94 ± 63	105* ± 17	51 ± 16	69 ± 1	51 ± 8		
	100	1.5	197 ± 71	276 ± 25	77 ± 21	66 ± 19	56 ± 3	46 ± 0		
	500	3.0			103* ± 40					
	100	3.0			53 ± 24					
	500	6.0			58 ± 19					
	100	6.0			62 ± 17					
Nonpolar neutrals	500	3.0	398 ± 13	224 ± 21	122* ± 7	107* ± 45	69 ± 5	53 ± 3		
	100	3.0	365 ± 7	279 ± 25	93 ± 9	88 ± 10	60 ± 7	37 ± 0		
Polynuclear aromatic hydrocarbons	500	1.5			1007* ± 159					
	100	1.5			394* ± 53					
	500	3.0			755* ± 144					
	100	3.0			283* ± 58					
	500	6.0			257* ± 198					
Positive control	100		2404* ± 218	280 ± 13	2309* ± 12	1848* ± 41	2086* ± 5	1260* ± 55	39 ± 20	174* ± 38
	10		2197* ± 682	1241* ± 215	2074* ± 31	1117* ± 47	1529* ± 13	623* ± 27	69 ± 20	20 ± 6
	1		1061* ± 14	627* ± 177	635* ± 38	109* ± 17	683* ± 30	339* ± 78	74 ± 6	20 ± 2
Solvent control	0.1 ml		218 ± 16	205 ± 9	51 ± 3	53 ± 5	64 ± 5	44 ± 3	38 ± 6	24 ± 6

[a]Plate-incorporation assays; results reported in mean ± standard deviation computed with triplicate plates.
[b]HA = metabolic activation.
[c]The polar neutrals and acids were tested in TA1535; however, the spontaneous revertants were not within acceptable limits.
*Mutagenic response is at least twofold that of the spontaneous revertants.

Table 5. Mutagenic, Carcinogenic, Neoplastic, and Teratogenic Compounds[a] Identified in Ambient Air

Compounds	Upland CA	Lake Charles LA (1)	Lake Charles LA (2)	Elizabeth, NJ	Beaumont, TX	Houston TX (1)	Houston TX (2)	Muta-genic	Carcino-genic	Neo-plastic	Tera-togenic
Acenaphthene				+						+	
Allyl-phenol	+									+	
Amino-anthracene	+							+		+	
Amino-anthraquinone	+					+				+	
Aniline		+							Indefinite		
Anthanthrene				+					+	+	
Anthracene	+	+	+	+		+			+	+	
Anthraquinone	+	+								+	
Benz-anthracene	+	+	+	+		+		+	+		
Benzo-fluoranthene		+	+	+		+			+	+	
Benzo-perylene			+	+					+	+	
Benzo-phenanthrene	+	+	+	+		+			+		
Benzo-pyrene	+	+	+	+		+		+	+		
Bromophenol		+								+	
Butylbenzylnitrosamine						+		+	+		+
Caffeine						+					+
Chlorophenol	+										+
Chrysene	+	+	+	+		+		+	±		
Cresol	+					+				+	
Dicyclohexylamine		+								+	
Dimethyl-anthracene						+				+	
Dimethyl-phenanthrene	+			+						+	
Fluoranthene	+		+	+		+				+	
Methylbenz-anthracene	+	+		+				+	+	+	
Methylbenz-phenanthrene	+	+	+	+				+	+	+	

[a]U.S. Department of Health, Education, and Welfare, 1977.

(continued)

Table 5. Mutagenic, Carcinogenic, Neoplastic, and Teratogenic Compounds[a] Identified in Ambient Air (continued)

Compounds	Upland CA	Lake Charles LA (1)	Lake Charles LA (2)	Elizabeth, NJ	Beaumont, TX	Houston TX (1)	Houston TX (2)	Muta-genic	Carcino-genic	Neo-plastic	Tera-togenic
Methyl-chrysene		+		+						+	
Methylene-phenanthrene	+									+	
Methyl-phenanthrene	+	+		+		+				+	
Methyl-pyrene	+	+		+		+				+	
Methyl-stearate	+		+	+		+				+	
Naphthacene	+	+							+		
Naphthalene	+	+				+				+	
Perylene	+	+	+	+		+			+		
Phenanthrene	+	+	+	+		+				+	
Phenol	+	+				+			+		
Phenyl-benzene				+						+	
Phenylene-pyrene				+					+		
Phenyl-β-naphythlamine	+			+		+		+	+	+	
Phenylphenol		+								+	
Progesterone				+					Suspected	+	+
Propenyl-phenol	+									+	
Pyrene	+	+	+	+						+	
Quinoline	+							+	+		
Styrene	+							±	±		
(p-Tetramethyl-butyl)phenol		+								+	
Tetrahydrobenzo-phenanthridine						+				+	
Toluidine						+			+	+	
Trimethyl-phenanthrene				+						+	
Triphenylethylene						+				+	

[a]U.S. Department of Health, Education, and Welfare, 1977.

Several problems were encountered in the development and validation of this technical approach to the investigation of the bioactivity of air particulate matter. Technical considerations concerning the specificity of the chemical fractionation scheme have been discussed previously (Pellizzari et al., 1978). The chemical fractionation scheme separates organic acids and bases by treatment with aqueous base and aqueous acid, followed by partitioning into organic solvent. Löfroth (1980) contends that such treatment may generate artifacts, especially in the organic base (nitrogen-containing compounds), due to reaction of substances with the aqueous acid or base during the extraction. Epler et al. (1978; Rao et al., 1980) recommended separation of the acid and base components with Sephadex LH-20 to avoid this problem; however, they found no significantly different results using LH-20 vs. acid-base extraction.

Sample size was a chronic problem in this study. The massive air volume sampler did not collect the amounts of particulate expected. In a 10-day sampling period, often less than 3 g particulate matter, including all three size fractions, was collected. Only 5 to 15% of the particulate represents extractable organics, and this material, when partitioned between five fractions, usually did not provide enough sample for quantitative dose-responsive plate-incorporation mutagenicity assays. For triplicate determinations in all five Salmonella strains, using five doses, both with and without metabolic activation (as suggested by deSerres and Shelby, 1979), the assay would require 50 mg. Enough material must also be available for chemical analysis. As can be seen from Table 3, none of the samples were of adequate size for complete bioassay and chemical analysis.

Despite the limited data available, the results of air particulate research by various investigators at different sites show certain similarities to this study. For example, Pitts et al. (1977) collected air particulate at rural and urban sites in California and extracted the organics using sonication with equal parts of methanol, benzene, and dichloromethane. The particulate collected from the rural site was not mutagenic, but material from all urban locations was mutagenic without S-9 addition, exhibiting a linear dose response for Salmonella strains TA98, TA1538, and TA1537. Thus, the mutagenic components present were probably not polynuclear aromatic hydrocarbons (PAH), which require activation. In another case, mutagenic activity requiring S-9 metabolic activation was demonstrated in a California air sample; this activity was then quantitatively related to B(a)P concentration (Flessel, 1980).

In Japan, ambient air was sampled at six residential and industrial sites (Tokiwa et al., 1977). The organic fraction from ambient air particulate (extracted with methanol) collected in

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Ohmuta (an industrial city) was mutagenic, exhibiting a linear dose response from 100 to 400 µg with Salmonella strain TA98. A sample collected from a residential area (Fukuoka City) required from 370 to 1970 µg/plate to generate a positive mutagenic response. The mutagenicity was approximately three times greater for the sample collected from the industrial city, and a linear dose response was observed for both samples. These researchers identified the compounds in the methanol extract using an alumina column and GC/MS analysis. A majority of the compounds identified were PAHs such as benzopyrenes, anthracenes, fluorenes, and dibenzoisomers of these compounds. These Japanese studies revealed that air particulate near industrial sites possessed higher mutagenic potential than that from rural sites and that both S-9-activated (PAH) and direct-acting (non-PAH) mutagenic components were present in air particulates, agreeing with the California studies of Pitts et al. (1977) and Flessel (1977).

Talcott and Wei (1977) collected air particulate on glass fiber filters and extracted the filter with acetone in a Soxhlet apparatus. The extracted material exhibited the highest activity with TA100 and required S-9 metabolic activation. Direct-acting mutagens were also detected with TA98 and TA1537. Activity was again attributed to at least two types of mutagens: a PAH and a non-PAH fraction. The view that PAH compounds are not the sole (or major) mutagenic factor associated with air particulate was further reinforced by the studies of Dehnen et al. (1977). Here, mutagenic activity was predominantly produced by compounds other than PAHs, since the observed mutagenicity did not require S-9. Dehnen used a chemical fractionation scheme to divide his crude air particulate sample into cyclohexane and methanol-extractable fractions. An alumina column was employed to further fractionate the cyclohexane extract into a purified cyclohexane fraction (containing PAH-type compounds) and a 2-propanol fraction (containing azo-heterocyclic compounds). The highest mutagenic response was found in the 2-propanol and methanol extracts; neither fraction contained PAH-like compounds.

Other investigators (Tokiwa et al., 1977) have combined a chemical fractionation scheme with a mutagenesis-detection system. After solvent extraction, fractionation of the crude organic extract into chemical classes permitted identification of the mutagenically active chemical compounds within each chemical class. In some cases, fractionation appeared to reduce or remove toxic effects that otherwise would have prevented expression of mutagenicity. Thus, the mechanism of synergism or antagonism of the individual components in the crude mixture is open to investigation. Pelroy et al. (1978) observed such effects with shale oil and its fractions, as did Rao et al. (1980) with fossil fuels and their fractions.

Pellizzari et al. (1978) initially tested a West Virginia ambient air particulate for mutagenicity using a fractionation scheme and the qualitative spot test (Ames et al., 1975) with the standard Ames Salmonella tester strains (TA98, TA100, TA1535, TA1537, and TA1538), both with and without a metabolic activation system (S-9). Mutagenic activity was observed for the organic bases, organic acids, and aromatics. Each of the five tester strains gave a positive response with at least one of the active fractions, and only the aromatics required metabolic activation (Hughes et al., 1978; Pellizzari et al., 1978). In this West Virginia sample, 2-nitro-4,5-dichlorophenol, a direct-acting mutagen (Pellizzari et al., 1978), was identified in the polar neutrals, and fluoranthene, pyrene, benz(a)anthracene, and B(a)P were identified in the aromatic fraction. All of these compounds have been identified either as co-carcinogens (Van Duuren, 1976) or as mutagens requiring metabolic activation (McCann et al., 1975).

These preliminary results indicated that the Ames assay could detect mutagenic activity in small sample amounts of ambient air (as a complex mixture), and that the fractionation and analysis scheme was capable of identifying broad chemical mutagenic classes within these mixtures. It was concluded that chemical fractionation of the crude complex sample was both useful and necessary to accomplish adequate biotesting and chemical identification of signature mutagenic components in ambient air particulate.

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